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# High Speed DNA Sequencing in Arrays of Glass Microchannels

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Most recent investigations of technologies for the next generation of high speed DNA sequencers have focused on rapid separations of DNA extension fragments in arrays of glass capillaries filled with low viscosity sieving media. These systems use small diameter glass capillaries (e.g. 50 um i.d., 250 um o.d.) that minimize the thermal temperature gradient across the sieving media due to joule heating so that larger electric fields can be used for more rapid separations than is typically used in most commercial DNA sequencers based on slab gel electrophoresis. Additionally, good sequencing quality separations (e.g. 500-800 bases in less than two hours) have been obtained using low viscosity polymer solutions that can be easily and quickly replaced by pressure filling techniques. Relatively large numbers of capillary array systems involving 96 to several hundred capillaries have been proposed or are under development.

We are investigating arrays of microchannels etched in a glass substrate as alternative technology to arrays of discrete glass capillaries. We have developed advanced microfabrication techniques that allow etching high density arrays of glass microchannels in large glass substrates and isolating the individual channels by glass fusion bonding of a second plate over the etched microchannel plate. This technology approach provides a number of advantages for building large arrays of electrophoresis microchannels for DNA sequencing. By fabricating the array of microchannels on a single glass substrate, the arrays of microchannels are very robust mechanically and can be handled without any special care. By means of photolithography and chemical etching techniques the dimensions of rectangular cross-section channels can be optimized by making the channel depth thin to minimize the thermal dispersion of DNA bands while at the same time the channel width can be made large to increase the amount of dye-labeled DNA available for strong fluorescence signal generation and detection. The detection of the fluorescence signal is also made easier by having a flat optical window over the channels through which laser excitation of fluorescence occurs with less scattered light of the primary laser beam to contribute to the overall noise level.

We have developed microfabrication technologies for producing high density arrays of microchannels of maximum lengths up to 42 cm long and are presently extending are capabilities to produce channel lengths up to 50 cm for a 96 channel array Channels of various cross section sizes have been built and tested using low viscosity formulations of linear polyacrylamide. Preliminary room temperature experiments for microchannels 44 um deep by 250 um wide show 300 bases resolved in less than 45 minutes. We expect this combination of microchannels and low viscosity sieving media to be able to detect more than 400 bases per channel in less than two hours time. We presently are building and assembling a 96 channel system based on this technology and will report on-going results obtained from it for high throughput DNA sequencing.

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